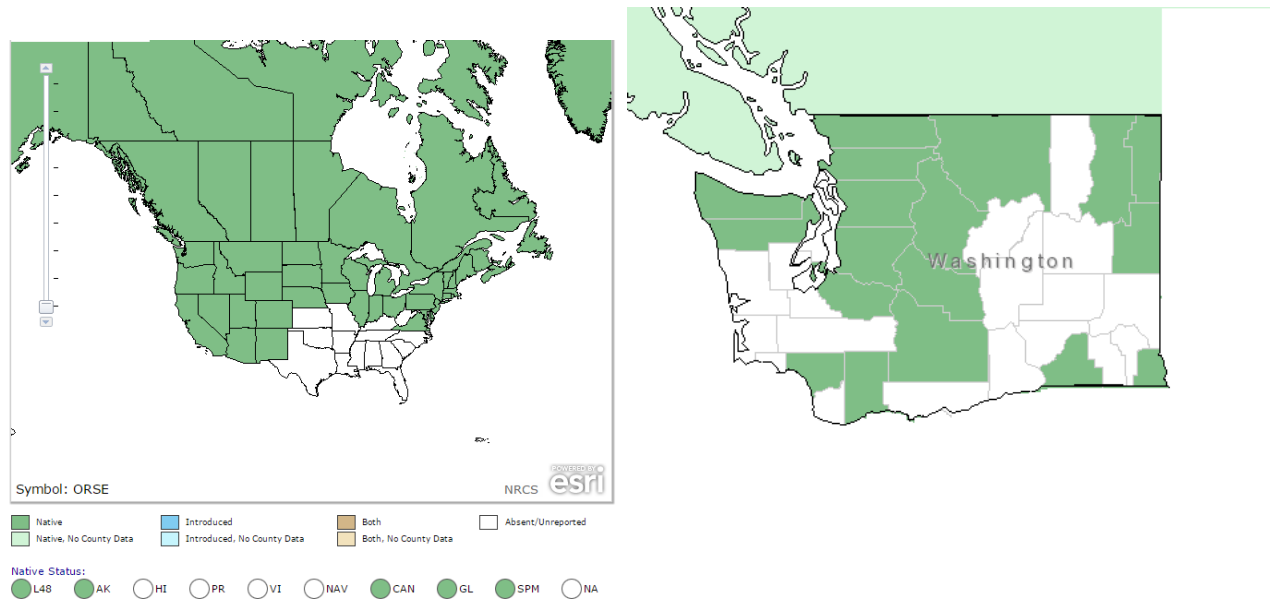


Plant Propagation Protocol for *Orthilia secunda* (*Pyrola secunda*)

ESRM 412 – Native Plant Production

Protocol URL: <https://courses.washington.edu/esrm412/protocols/ORSE.pdf>



TAXONOMY

Plant Family	
Scientific Name	Pyrolaceae
Common Name	Shinleaf
Species Scientific Name	
Scientific Name	<i>Orthilia secunda</i> (L.) House
Varieties	<i>Orthilia secunda</i> (L.) House var. <i>obtusata</i> (Turcz.) House
Sub-species	<i>Orthilia secunda</i> (L.) House ssp. <i>obtusata</i> (Turcz.) Bocher
Cultivar	None specified
Common Synonym(s)	<i>Pyrola secunda</i> (L.), <i>Pyrola secunda</i> var. <i>obtusata</i> (Turcz.) ¹¹ , <i>Pyrola secunda</i> ssp. <i>obtusata</i> (Turcz.) ¹¹ , <i>Ramischia elatior</i> Rydb. ¹¹ , <i>Ramischia secunda</i> (L.) ¹¹
Common Name(s)	Sidebells wintergreen, one-sided pyrola, one-sided wintergreen, one-sided shinleaf, one-sided pryola.
Species Code (as per USDA Plants database)	ORSE or PYSE (former code)
GENERAL INFORMATION	
Geographical range	See above
Ecological	Found in moist forests and bogs ¹⁰ ; upland forests of the dry, dry-mesic

distribution	and mesic varieties ⁵ . Is typically found in mixed conifer forests ⁹ . Can sometimes be found in old meadows, and some small stands can be found along roadsides ⁷ . Prefers environments with low to moderately acidic soils ³ . Is common throughout the region – the only noted location where it is rare is British Columbia’s Queen Charlotte Islands ¹² .
Climate and elevation range	Found at upper elevations ⁹ . Found throughout the Pacific Northwest, ranging north to the Arctic coast ¹³ .
Local habitat and abundance	Prefers shade, so is often found on the shadows of the forest floor; however, some plants have been found in locations where they seem to be “seeking out the light.” Often grows in clumps ⁷ .
Plant strategy type / successional stage	Competitor – can withstand the dark, cool, moist areas of the forest floor, yet also tolerates low light in some locations ⁷ .
Plant characteristics	<p>General: Evergreen, stem arises from a slender rhizome². Considered a sub-shrub, and usually reaches a height of 10-30 cm. Has very thin, long rhizomes that can grow up to 1 m. in length³.</p> <p>Leaves: Round to elliptical in shape. Found along plant in separated clusters¹⁰; simple leaves, some basal arrangement⁵, some cauline⁹. Plant is known for its leaves that remain green throughout the winter, which is why many of its common names include “wintergreen.” (Lady Bird wildflower)</p> <p>Fruit: Rounded, nodding capsules with 4-5 lobes. Stems are erect during the fruiting stage of the plant⁷. Fruit often not mature until late summer/early fall¹⁵.</p> <p>Flowers: White to light-green in color; 5 parted, stalked. Petals are 1/3 inch long, protrude at an angle. Inflorescence is a cluster⁵ located on only one side of the plant¹⁰. Bloom between June and August¹⁴.</p> <p>Ethnobotany: The Bella Coola Tribe of British Columbia reportedly used the root of the plant as an ingredient in an eyewash liquid medication⁸. Many Pacific Northwest First Nations mash the leaves of the plant and use it in salves or poultices to help treat skin eruptions – the acids in the leaves are helpful in bringing down inflammation in the skin¹².</p>
Propagation of <i>Orthilia secunda</i> by V.A. Johansson and O. Eriksson assessing success of germination in terms of proximity to adult plants and soil nutrient/fungal levels⁶	
Ecotype	Seeds were collected in three different regions of Sweden: Oland, Uppland, and Sodermandland. Sites were all dry – mesic mixed conifer-deciduous forests that featured rather open canopies (canopy cover not recorded in study) and had a diverse understory.
Propagation Goal	Plants - Germinants
Propagation Method	Seed
Product Type	No commercial product – not applicable for this study.
Stock Type	Native seed collected from three sites (see above)
Time to Grow	Eight months or 12 months was the germination period. The germinants were discarded after experiment was completed; no outplanting was

	performed beyond the experimental in-plot germination.
Target Specifications	Successful germinants – size and characteristics beyond germinating not defined by researchers.
Propagule Collection Instructions	Seeds were collected in September 2009 from mature fruiting <i>Orthilia secunda</i> capsules that were either already releasing seed or about to release them. All seeds from each mother plant (approximately 50 seeds per packet, 3 packets per bag for about 150 per bag) were packed in bags made from 10 mm by 40 mm rectangles of 50 um mesh size plankton cloth. These bags were chosen to allow fungal hypha to pass through the cloth. After seeds were in the bags, all were sealed with a heat sealer.
Propagule Processing/Propagule Characteristics	Mean seed size was $19 \times 10^{-4} / \text{mm}^3$. Seed longevity was not tested in this research.
Pre-Planting Propagule Treatments	No cleaning or storage – plants were collected and then immediately planted.
Growing Area Preparation / Annual Practices for Perennial Crops	Both control and experimental plots were designed in the seed collection areas. These plots were built in both areas where <i>Orthilia secunda</i> adult plants were not found (these were the unoccupied control plots, located at least 100 m. from center of experimental plot) as well as areas where the adult plants were found. Five different sites with both control and experimental plots were built. Eighteen seed bags (of approximately 150 seeds) were buried in each plot.
Establishment Phase Details	<p>Seeds were buried in the surface of the soil of each test plot (both unoccupied plots and those with adult plants present) in September 2009. Bags were attached to a nylon string that was left above the soil surface; this string was then tied to a nail for easy retrieval of the bags.</p> <p>Half of seeds were taken out from their burial locations in the plots in June 2010 and half were taken out from burial in September 2010. Seed bags were then transferred to moist plastic bags and stored at a constant temperature of 4 deg. C for 3 days.</p> <p>Two seed packets from each plot were mounted on glass slides prepared with 50% glycerol with cover slips; the third packet was used for molecular sampling that is the focus of another research study.</p> <p>Germination rates of packets were analyzed using microscopes enabled with cameras connected to a computer. All seeds and germinants were counted; the fraction of germinants was calculated for each packet. All packets showed signs of fungal growth and fungal hyphae, so this was not included in the analysis.</p> <p>Germination was found to be much higher in plots with adult <i>Orthilia secunda</i> plants present – an average of 13.7% of seeds germinated in plots with adults, while only 3.4% of seeds in control plots germinate.</p>

	<p>Seedling size was also, on average, larger in plots with adult plants. Bags of seed taken during the harvest in September 2010 warranted a much higher germination rate than the bags unearthed in June 2010. Soil nutrient differences and pH did not make a statistically significant difference.</p> <p>Each microsite also produced an effect in the <i>Orthilia secunda</i> germination rates – while other species that were studied showed mix of seed limitation and microsite limitations, <i>Orthilia secunda</i> is almost exclusively limited by microsite conditions. Since nutrient levels did not show an effect, the fungal types and conditions are hypothesized to be the main contributor to this effect on germination; however, more research needs to be conducted to confirm this.</p>
Length of Establishment Phase	8 months or 12 months.
Active Growth Phase	Not part of the study.
Length of Active Growth Phase	Not part of the study.
Hardening Phase	Not part of the study.
Length of Hardening Phase	Not part of the study.
Harvesting, Storage and Shipping	Not part of the study.
Length of Storage	None, not part of the study.
Guidelines for Outplanting / Performance on Typical Sites	<p>Propagating <i>Orthilia secunda</i> seeds directly through sowing in sites can be successful, given that the right microsite conditions exist. Propagation in a greenhouse setting would likely be more successful if seeds are planted in pots or on flats that already have adult <i>Orthilia secunda</i>, as this would likely prompt higher rates of success. Having adult plants present in a propagation environment in a greenhouse may also better guarantee that microsite conditions are in an acceptable range for the seeds to germinate. A. Huxley and M. Griffiths echo this statement, saying that seeds can be difficult to propagate for <i>Orthilia secunda</i>, and that plants should be sowed near an adult plant or potted with soil that's come from an area adjacent to an established plant to capture the correct mycorrhizae⁴.</p>
Other Comments	None at this time.
<p>Genetic analysis of <i>Orthilia secunda</i> by G.E. Beatty et al assessing success of possible future propagation based on population location (edge vs. center of population)¹</p>	
Ecotype	<p>Arctic and subarctic locations in Sweden, Scotland and Ireland. Scottish Highland locations: Glen Glass, Black Isle, Little Scatwell, Tor Schilty (all four main range populations); Glen Mhor, Glen Banvie (both range edge populations). All locations are either pine woodlands or birch</p>

	<p>woodlands.</p> <p>Swedish locations: Flurmark (range edge population) and Lomselenas (a peripheral population); both spruce/birch woodlands.</p> <p>Irish locations: Lough Navar scarplands (a sandstone plateau) and Cranny Burn a mixed pine/birch woodland. Both locations in Ireland were peripheral populations.</p>
Propagation Goal	This study examined genetic variation assessment to aid in future plant harvests to maintain high genetic diversity in <i>Orthilia secunda</i> populations. Future goals of this work include stronger plants for the future.
Propagation Method	Vegetative – single leaves were taken from larger rosettes only when it was possible to remove the leaf without damaging the plant.
Product Type	Genetic DNA to assist with future seeding efforts.
Stock Type	Native <i>Orthilia secunda</i> leaves taken from several locations in Northern Europe (see above).
Time to Grow	Not examined for this study.
Target Specifications	Work done to promote wide genetic diversity among future propagules.
Propagule Collection Instructions	Leaf samples were collected in the summer. Between 19 – 26 leaves were collected from each patch area; leaves were collected from individual rosettes in semiregular intervals throughout each patch. Patch areas ranged from 2m by 2m to 20m by 20 m, and were based off of natural populations. A population was designated as a location that was separated from another patch of <i>Orthilia secunda</i> by at least 20 meters; some patches were as far as 3000 meters from each other. Both main range plant populations and edge populations had similar numbers of samples collected.
Propagule Processing/Propagule Characteristics	Not examined for this study.
Pre-Planting Propagule Treatments	<p>Samples were stored at -20 deg C in separated plastic bags to retain leaf moisture.</p> <p>Plant samples were then ground for 8 minutes at 30 Hz in order to better visually express DNA of the plants. DNA was extracted from samples that had been diluted to a concentration of 50 ng/uL on 1% agarose gels stained with ethidium bromide. DNA was quantified using polymerase chain reaction (PCR) methods. Nuclear microsatellite isolation and microsatellite genotyping was also conducted to further separate genetic code distinctions and examine plant population differences.</p>
Growing Area Preparation / Annual Practices for Perennial Crops	No growing area was prepared for this study; however, results from this study are expected to inform sample collection for propagation of <i>Orthilia secunda</i> .
Establishment Phase Details	Not part of this study. See above for plant sample genetic extraction methods.

Length of Establishment Phase	The experimental design does not provide a good estimate for this.
Active Growth Phase	Not part of this study.
Length of Active Growth Phase	Not part of this study.
Hardening Phase	Not part of this study.
Length of Hardening Phase	Not part of this study.
Harvesting, Storage and Shipping	Not part of this study.
Length of Storage	Not part of this study. The only storage of leaves was conducted at the beginning of the experiment after collection.
Guidelines for Outplanting / Performance on Typical Sites	Results of the genetic analysis showed that the periphery collection locations in Ireland had significantly higher genetic diversity than the rest of the study locations – all other sampling sites showed similar levels of genetic diversity, regardless of population location (main, edge or periphery). Irish sampling spots were also the sites at the lowest latitude in the study. This demonstrates that <i>Orthilia secunda</i> populations at latitudes lower than 55 N may be better to collect from for propagation processes which have a main objective or retaining genetic diversity, at least in Europe – additional studies focusing on other geographic populations are necessary to examine genetic diversity and make collection recommendations. However, given that <i>Orthilia secunda</i> is difficult to propagate from seed, further investigation is needed.
Other Comments	Not at this time.
INFORMATION SOURCES	
References	See below
Other Sources Consulted	See below
Protocol Author	Ashley Blazina
Date Protocol Created or Updated	May 20, 2015

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